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JAPAN REPORT

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BIOTECHNOLOGY

DNA, CHROMOSOME ENGINEERING POTENTIAL IN PLANT BREEDING SEEN

Tokyo BIO INDUSTRY in Japanese Nov 85 pp 54-63

[Article by Masanobu Mino of the Center for Agricultural Technology, National Federation of Agricultural Cooperative Associations]

[Text] In the field of breeding, it is often desirable to introduce a large number of genes efficiently and all at once. Although there are remarkable development in vector systems including the Ti-plasmid, the simultaneous introduction of a large number of genes has not been achieved. In that sense, the technological development for introducing DNA or chromosomes into protoplasts is highly valuable: its application is also conceivable to the production of useful substances involving a large number of genes.

1. Introduction

It has been over 20 years since transformation research began in plants, and remarkable advancements have been made recently associated with the development of various vector systems. Of those, the most advanced technique is genetic recombination using the Ti-plasmid. However, owing to the isolation of protoplasts and the development of culture techniques, the accumulation of various new findings and techniques have been reported regarding the non-specific DNA incorporation system which has been

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<>In this article, DNA engineering denotes the introduction of foreign DNA including genetic recombination using vectors, and chromosome engineering denotes introduction of foreign chromosomes. However, conventional cross breeding techniques are excluded.

attempted since the onset of transformation experiments. One example is the accurate incorporation of a large amount of genetic information into a cell made possible by the use of liposomes and a microinjection technique.

On the other hand, at the actual breeding site, one must frequently deal with characters controlled by a large number of genes as the breeding goal target. They are called quantitative characters, encompassing many economic characters such as yield. When thinking about the application of transformation techniques to breeding under such circumstances, I believe that the current transformation system using vectors has not lost its importance as a potential technique for introducing a large quantity of genetic information since the simultaneous introduction of a large number of genes has not yet been achieved. In view of the above-mentioned points, I shall discuss in this article the potentials of as well as the actual technologies available for transformation systems using DNA or chromosome introduction to cope with the limitations faced by current breeding technology.

2. Limitations of present breeding techniques

In the case of breeding techniques currently used, the majority of them require manual crossing for the introduction of useful genes. Consequently, even though the breeder wishes to proceed with breeding according to his desires, the following three points at times cause problems. First, the problem relates to the lack of affinity in interspecies and intergeneric cross breeding; the second is the difficulty of cross breeding vegetatively propagated plants. In other words, these problems are reproduction problems and complex genetic mode problems. The third problem is how quickly the non-target gene group can be eliminated in the hybrid progeny to establish genetic stability as a strain, which involves cross breeding in general. The first problem is attributable to low rate of crossability among distantly related species. Techniques to overcome this problems include the use of bridging plants, ovule, ovary, and embryo culture. However, if the species are more remotely related, these techniques are ineffective. The second problem is attributable to the fact that character recombination is often difficult in hybrid progeny of vegetatively propagated plants since the majority of them are hetrozygotes. This fact causes a markedly lower breeding efficiency in breeding programs designed to endow new disease resistance while maintaining the superior characteristics of the extant species. Consequently, the use of artificial mutation is believed to be the most effective way. However, the shortcoming is that even with the use of various mutagenic substances, the control of directed mutation is currently impossible, and as a result, an enormous number of individuals must be treated to obtain an individual having the

desired characteristics. The third problem relates to time limitations and breeding scale. If the characters sought are controlled by a small number (one in some cases) of major mobile genes such as disease resistance, breeding can proceed in a short period of time by handling a relatively small number of individuals in continuous back crosses. However, in the case of characters controlled by slightly mobile genes such as yield, component contents and environmental stress resistance such as cold resistance, it becomes necessary to increase the population size handled depending on the number of genes involved or adopt a long-term selection plan. In the case of self-propagating plants, labor and time can be reduced or shortened to a certain extent by introducing generation acceleration and population breeding techniques using environmental control facilities, but they also have limitations.

In summary, the limitations of the present breeding techniques will appear 1) when the desired genes cannot be found in the crossable classification groups, 2) when one or two new characteristics must be added urgently to existing varieties of vegetatively propagated plants, and 3) when genetic stability of quantitative characters is sought urgently. On the other hand, in the case of transformation systems, 1) if proper promoters can be introduced simultaneously, the distance from the genetic origin does not matter; 2) when the character introduced is controlled by a small number of genes, which can be selected in vitro, breeding efficiency can be increased; and 3) even when the character is controlled by a large number of genes, if only the chromosomes involved are introduced, the mixing of undesirable gene groups can be mostly avoided, which is advantageous in improving selection efficiency. For these reasons, it is desirable to develop new breeding techniques that allow the rapid and steady introduction of desired genes (gene groups) into the individuals to be improved.

3. Transduction systems for foreign genes

The transduction systems for foreign genes presently known are summarized in Fig. 1. No explanation is required for the cross breeding technique; and since chromosome engineering using heteroploids is outside the scope of this article, reference should be made to other sources[1)]. Furthermore, the transduction system for specific DNA using vectors and the cell fusion technique will not be discussed here since they are explained in other articles in this series. Regarding techniques other than those mentioned above, I shall divide them into DNA engineering techniques and chromosome engineering techniques and introduce the substance of these techniques in order.

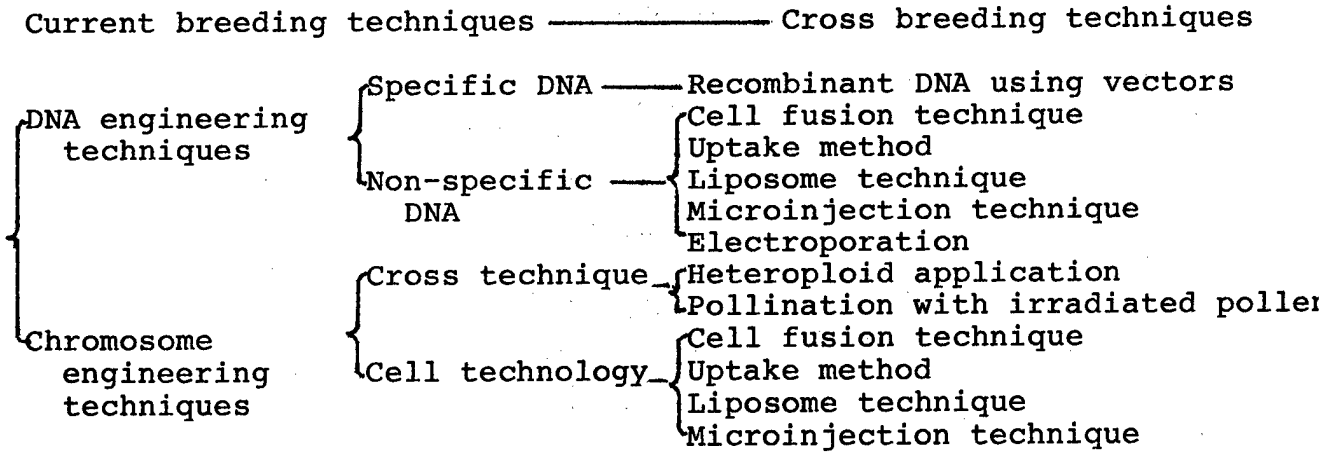

Fig. 1 Transduction systems for foreign genes

3.1 DNA engineering techniques

3.1.1 Uptake method

For the transformation system to accomplish transduction of foreign DNA, 1) the DNA must be incorporated into the recipient cell without being degraded, 2) it must replicate itself as the recipient cell divides, and 3) it must be expressed[2]. Of these, the most important thing is to introduce the donor DNA into the target individual or cell without damaging it by protecting it from degradation by the DNase in the recipient cell. The treatment of plant parts with foreign DNA resulted in many cases with the expression of donor characteristics in the immediate and subsequent generations following treatment (Table 1). Hess[3] reported that he immersed white petunia buds for 15 minutes in a DNA solution (0.1 mg/ml) extracted from red flowering plants and further allowed the roots to absorb the solution for 4 hours, which resulted in 27 percent of the treated plants to change to red flowering plants with the character expressed in subsequent generations. In view of the fact that when a similar treatment with the DNA of white flowering plants was used, the red flowers were expressed in 9 percent. It is believed that a donor DNA effect certainly exists. Similarly, regarding late blight resistance of potato[4] and the auxotrophy of Arabidopsis[5], there are reports of transformation following DNA treatment of respective leaves and seeds. However, in these reports, the form in which the donor DNA was incorporated and how the DNA reached the nucleus to be expressed are unknown.

Table 1 DNA-uptake method in plants

(1) 材料（特性）	(2) 外来DNAの種類	(3) 処理条件 (4) 処理法	(5) 時間(h)	温度(℃)	(6) 取込みの検定と形質発現	(7) 文献
(8) ペチュニア（白花）	(9) 赤花ペチュニアのDNA	(10) DNA溶液への芽生の浸漬	4.25		(11) 赤花個体が出現	3)
(12) ジャガイモ（疫病親和性）	(13) 疫病不親和性雑種のDNA	(14) DNA溶液の葉身への塗布	←		(15) — 抵抗性反応が見られた	4)
(16) アラビドプシス（栄養要求性）	(17) 細菌DNA，子牛の胸腺DNA	(18) DNA溶液への種子の浸漬	96		(19) 最少培地でも発芽した	5)
(20) タバコ葉肉プロトプラスト	^{14}C-fd-DNA（二重鎖）	(22) 2×10^6プロトプラスト/ml +2.5－10 μg DNA/ml +5 μg PLO*/ml +5 mM Zn^{2+}	1	25	(23) TCA不溶画分/全取込み	6)
(24) ササゲ葉肉プロトプラスト	^{3}H-pBR 313 DNA	(22) 1×10^6プロトプラスト/ml +3－4 μg DNA/ml + 5 μg PLO/ml +5 mM Zn^{2+}	0.25	25	アガロースゲル (25) 電気泳動法	9)
(26) タバコ培養細胞プロトプラスト（cv. Xanthi）	(27) タバコ（cv. Xanthi nc）^{3}H-DNA *E. coli* ^{3}H-DNA	(22) 4×10^6プロトプラスト/ml +2.1 μg DNA/ml + (28) DEAEデキストランまたは PLL** 5 μg/ml	1－5	27	TCA沈澱，DNA/DNA雑種形成法	8)
(30) オオムギ葉肉プロトプラスト	*B. subtilis* －^{3}H-DNA, *M. lutes* －^{125}I－DNA	(22) 1.8－4.6 $\times10^6$プロトプラスト/ml +4.5－7.5 μg DNA/ml	6	27	(31) 全取込み量	10)
(20) タバコ葉肉プロトプラスト	^{3}H－λ－DNA（二重鎖）	(32) 2×10^6プロトプラスト/ml + 5 μg PLO/ml +5mM Zn^{2+}	1	25	TCA不溶性画分／全取込み	7)
(33) オオムギ，タバコ葉肉プロトプラスト	^{3}H－pBR 313DNA	(34) PLO, Zn^{2+}, カイネチン，アルカリ（pH9.0）	2－6	26	セファロース (35) 4Bカラムクロマト	11)
(36) ダイズ培養細胞プロトプラスト	^{3}H－pBR 322DNA	(22) 1×10^6プロトプラスト/ml + 5 μg PLO/ml	0.5	33	TCA沈澱 (37)	12)

*, **, PLO, PLL ; Poly-L-ornithine, Poly-L-lysine

key: 1) material (characteristic)
2) type of foreign DNA
3) conditions for treatment
4) method of treatment
5) time (h), temperature (°C)
6) incorporation test and gene expression
7) literature
8) petunia (white flower)
9) DNA of red petunia
10) immersing buds in DNA solution
11) red flowering plant expressed
12) potato (late blight affinity)
13) DNA of late blight non-affinity hybrid
14) coating leaves with DNA solution
15) resistance reaction was observed
16) Arabidopsis (auxotrophy)
17) bacterial DNA, calf thymus DNA
18) immersing seeds in DNA solution
19) germinated even in the minimal medium
20) tobacco leaf mesophyll protoplast
21) ...(single stranded chain)
22) protoplast

23) TCA insoluble fraction/total incorporation
24) green bean leaf mesophyll protoplast
25) agarose gel electrophoresis
26) cultured tobacco cell protoplast 27) tobacco...
28) TCA precipitation, DNA/DNA hybridization
29) DEAE dextran or ... 30) barley leaf mesophyll protoplast
31) total amount of incorporation
32) ...(double stranded chain)
33) barley, tobacco leaf mesophyll protoplast
34) ...kinetin, alkali...
35) sepharose 4B column chromatography
36) cultured soy bean cell protoplast 37) TCA precipitation

Suzuki and Takebe[6] elucidated the fact that phage fd-DNA (single stranded chain) incorporation into the tobacco leaf mesophyll protoplast is markedly enhanced by poly-L-ornithine (PLO) and Zn^{2+}. They incubated ^{14}C-fd-DNA (2.5-10 µg/ml) and protoplast (2.6×10^6/ml) at 25°C for 1 hour, removed the DNA adhered to the protoplast surface by DNase treatment, and measured the radioactivity incorporated into the protoplast. In that case, when PLO (MW. 1.3×10^5, 5 µg/ml) was added to the medium, as much as 6-fold incorporation was found compared to the control. A similar effect was also found with poly-L-lysine with about a 5-fold increase compared to the control. Although a PLO effect was also found when 10 µg/ml or more was added, in this case, contraction and agglutination phenomena of the protoplasts occurred. This fact revealed that it was not specific incorporation that increased, but non-specific adsorption of DNA to denatured protoplasts occurred. On the other hand, the addition of Zn^{2+} (5mM) markedly increased incorporation without causing denaturation of the protoplast. Cu^{2+} was also effective, but the effect was due to protoplast denaturation. In conclusion, they stated that it is desirable to incubate protoplasts in the fd-DNA (up to 30 µg/ml) solution (neutral, 25°C) containing 5 µg/ml PLO and 5 mM $ZnSO_4$. Under these conditions, 10-30 percent donor DNA was incorporated into the protoplast, 60-80 percent of which was in the cytoplasmic fraction, and about 30 percent of that maintained its original molecular weight. In a similar subsequent experiment using lambda phage-DNA (double stranded chain), it was discovered that treating DNA in advance with PLO was more effective[7]. The reason that PLO enhances DNA incorporation is believed to be due to the fact that positively charged PLO and DNA are readily bound and form a structure that is resistant to DNase. The incorporation of poly-L-lysine and DEAE dextran is probably promoted by a similar mechanism[6),8)]. On the other hand, the

actions of Zn^{2+} and Fe^{2+} are believed to be their mediation of DNA-PLO adsorption on to the protoplast surface[7], and as a result, undecomposed DNA is incorporated inside, perhaps increasing the chance of their reaching the nucleus.

The effect of PLO to promote incorporation has been found in other materials as well[9)-10)], and the effect can be further enhanced by selecting an appropriate time for treatment. Cress[12)] synchronized the cell division cycle of cultured soy beans using FUdR and thymidine treatment, prepared protoplasts, and observed the incorporation of ^{3}H-pBR322 in the presence of PLO at various times following FUdR removal. As a result, it was found that incorporation during the S phase is increased 2.5-4.5 fold compared to the G_1 and G_2 phases. A similar finding was obtained at the time of TMV-RNA transduction using liposomes[13)].

3.1.2 Liposome technique

The transduction of foreign genes mediated by liposomes has been well studied in mammalian cells[14)-16)]. Wong, et al.[15)] removed 875 base pairs of DNA fragments coding for beta-lactamase from E. coli plasmid pBR322 and reported that when, after encapsulating them in liposomes and incubating them along with HeLa and CEC cells, beta-lactamase activities not found in eukaryotes were detected in the sample cells, and this property was expressed through at least several cell cycles. Schaffer-Ridder, et al.[16)] also reported that when a recombinant plasmid pAGO coding for thymidine kinase (TK) was introduced into a murine L cell (TK^{-}) mediated by a liposome and cultured in HAT medium, colonies of stable transformants were formed at the frequency of 0.02 percent.

On the other hand, although research cases of plants have been increasing in recent years, with the exception of transfection studies[13),17)] using viral genes, the expression of transduced genes has not been confirmed (Table 2). Uchiyama and Harada[18)] encapsulated ^{3}H-pBR322 DNA in a reverse-phase evaporation lipid vesicle (REV) prepared from a mixture of lecithin (phosphatidyl choline) and stearylamine (molar ratio of 1:3), incubated them with cultured soy bean cell protoplasts to develop 3.2×10^{-7} μmol lipid/protoplast and analyzed the DNA incorporated by agarose gel electrophoresis. The result revealed that 11.8 percent of the donor DNA was incorporated into the cell, of which open circular DNA disappeared as time passed, whereas the covalently closed circular (ccc) DNA was stably maintained.

Table 2 Incorporation of genetic information using liposomes in plants

(1)材料（特性）	(2)外来DNAの種類	(3)処理条件 (4)処理法	(5)時間(h)	(6)温度(°C)	(7)取込みの検定	(8)文献
(9)ニンジン培養細胞プロトプラスト	^{3}H-pBR322DNA	3.2～10 μmol lipid/プロトプラスト (10)	1	25	アガロースゲル (11)電気泳動法	18)
(12)タバコ葉肉プロトプラスト	^{3}H-pBR322DNA	100nmol lipid/1×10^{6}プロトプラスト (10)	3	25	(13)セファロース4Bカラムクロマト	20)
(14)タバコ培養細胞プロトプラスト	TMV-RNA	2 μmol lipid/プロトプラスト (10)	0.25		(15)蛍光抗体法	13)
(16)ニンジン培養細胞，ササゲ葉肉プロトプラスト	pBR322等 (etc.)	lipid/プロトプラスト比は様々 (17)	0.25	22	オートラジオグラム等 (18)	19)
(19)ササゲ葉肉プロトプラスト	CCMV-RNA	lipid/プロトプラスト比は様々 (17)	0.25	25	(15)蛍光抗体法	17)

key: 1) material (characteristics)
2) type of foreign gene
3) conditions for treatment
4) method of treatment
5) time
6) temperature
7) incorporation test
8) literature
9) cultured carrot cell protoplast
10) protoplast
11) agarose gel electrophoresis
12) tobacco leaf mesophyll protoplast
13) sepharose 4B column chromatography
14) cultured tobacco cell protoplast
15) fluorescent antibody technique
16) green bean leaf mesophyll protoplast, cultured tobacco cell protoplast
17) lipid/protoplast ratio varies
18) autoradiogram
19) green bean leaf mesophyll protoplast

The type of liposome also greatly influences the efficiency of transduction. Lurquin and Sheehy[19] proved that there are big differences in protoplast adsorption among several kinds of multilamellar liposomes (MLV) prepared by alternately adding lecithin and stearylamine or without addition, or the liposomes made with phosphatidyl serine. However, they stated that adsorption was high and showed virtually no difference between the positively charged MLV (lecithin/stearylamine) and negatively charged REV (phosphatidyl serine/cholesterol) in spite of the fact that the protoplast surface is negatively charged.

The timing for liposome treatment is also important. According to Nagata, et al.[13], in the case of TMV-RNA transfection of cultured tobacco cell protoplast via liposomes, infection occurred at a 37 percent level during the M phase and 26 percent during the S phase when the cell division cycle is synchronized by amphidicholine treatment (5 mg/l). This finding makes us

expect that foreign genes transduced into the cytoplasm are readily incorporated into nucleus during the M phase due to absence of a nuclear membrane. On the other hand, one should also be aware that the lipid/protoplast ratio[20] and type of buffer[18] affect protoplast activity.

The advantages of using liposomes in the transduction system for foreign genes are 1) degradation due to DNase of protoplast origin can be minimized and 2) due to their high affinity for the cell membrane, they enhance the chance of transduction of the adhered foreign genes into the cell. On the other hand, they have a shortcoming of decreased capacity to encapsulate when the molecular weight of DNA is larger. As the molecular weight exceeds $1x10^6$ daltons, that capacity decreases suddenly[21], and large fragments of DNA cannot be transported. Conversely, there is a method[22] of preparing large liposomes having diameters of 10-100 μm (normally, large one are about 1 μm).

3.1.3 Microinjection technique

In the case of the uptake method and the liposome technique, the DNA introduced through the cell membrane is exposed to nucleases in the cytoplasm before reaching the nucleus making it difficult to accurately induce the desired amount of DNA into the nucleus. On the other hand, the microinjection technique allows direct injection into the nucleus of the target cell under a microscope preventing cytoplasmic degradation. In addition, it is possible to transduce the desired amount of DNA since the amount of injection is controllable. This technique was also developed mainly with mammalian cells, and because of longer studies on DNA injection into oocytes or ova and their expression, the accumulation of knowledge is greater in animals than plants[23)-30].

Anderson, et al.[25] injected a recombinant plasmid xl having the TK gene of HS virus and pRK 1 having a human beta-globulin gene into mouse fibroblasts (TK^-) and cultured them in HAT medium. Subsequently, when the cells which formed colonies in the medium were studied, TK activities were found in them, and at the same time, though in a small amount, human beta-globulin mRNA was also found. These data demonstrate the fact that the DNA injected are biologically functioning. Wagner, et al.[27] injected rabbit beta-globulin genes consisting of 6,200-base-pair DNA fragments into the fertilized egg of a mouse and transfered it into the womb of another mouse and had it deliver. The results not only confirmed the presence of the rabbit genes in the grown animal but revealed that the globulin product is also synthesized and

even transmitted to the offspring. Frels, et al.[30] injected pig histocompatibility genes into a fertilized mouse ovum and confirmed the expression of the transduced genes in the treated generation and progeny by immune and transplantation rejection responses. In addition, injected mammalian or viral genes were also frequently expressed in amphibians[28]. The replication and expression of genes between phylogenetically distant species following transduction is defined especially as "transgenosis." The success rate of transgenosis by microinjection in animals is probably related to how readily injection can be done into oocytes or ova.

On the other hand, the first example of transformation in plants by microinjection is perhaps the experiment with barley by Soyfer[32),33)] (Table 3). According to his report, when DNA extracted from the seeds of milk-ripe stage two-rowed barley (non-glutinous type) was injected into the seeds of milk-ripe stage six-rowed barley (glutinous type), the donor characteristics were expressed in individuals of the treated generation down to the selfed fourth generation progeny. However, only the DNA of the milk-ripe stage seeds were effective in transformation.

Table 3 DNA microinjection technique in plants

(1) 材料（特性）	(2) 外来のDNAの種類	(3) 処　理　法	(4) 取込みの検定と形質転換	(5) 文献
(6) 六条オオムギ（モチ性）	(7) 二条オオムギ（ウルチ性）の乳熟期の種子からのDNA	(8) 乳熟期の種子に注入	(9) 花粉のウルチ性反応，二条性などが発現	33)
(10) タバコ葉肉プロトプラスト	−	(11) Polylysineをコートしたガラス面にプロトプラストを固定し，20%Lucifer Yellow溶液を顕微鏡下で注入	(12) 蛍光顕微鏡観察	36)
(13) ハナキリン培養細胞プロトプラスト，タバコ葉肉プロトプラスト	(14) サケの精巣DNA	(15) 捕捉ピペットでプロトプラストを固定し，Lucifer Yellow又はベルベリン−DNAを顕微鏡下で注入	(12) 蛍光顕微鏡観察	38)

key: 1) material (characteristics) 2) type of foreign DNA
3) method of treatment
4) incorporation test and transformation
5) literature 6) six-rowed barley (glutinous type)
7) DNA from the seed of milk-ripe stage of two-rowed barley (non-glutinous type)
8) injected into milk-ripe stage seeds
9) non-glutinous character response of pollen, two-rowed character expressed

10) tobacco leaf mesophyll protoplast
11) protoplast immobilized on a glass surface coated with polylysine and injected with a 20 percent lucifer yellow solution under a microscope
12) fluorescence microscopy
13) protoplast of cultured Euphorbia millii cell, tobacco leaf mesophyll protoplast
14) DNA from salmon seminal vesicle
15) immobilized the protoplast with a trapping pipette and injected lucifer yellow- or berberine-DNA under a microscope

In order to transduce DNA accurately into the nucleus, it is still necessary to inject it directly into the protoplast. The injection is manipulated under a microscope, which requires ingenuity for a smooth process in two aspects. The first is the technique of immobilizing the protoplast; and the second is the method for verification of injection. Plant protoplasts, unlike animal cells, do not have the property to adhere to a glass surface. Consequently, one must device a way to immobilize them with alginic acid[34),35)], polylysine[36)], or agar. Furthermore, in the case of animal cells, injection can be verified by the granular flow in the cytoplasm or changes in light refraction[26)], which is difficult in plant cells due to presence of vacuoles, and it is necessary to use dyes harmless to the cell as markers. Steinbiss and Stabel[36)] reported that they immobilized a day-old tobacco leaf mesophyll protoplast culture with polylysine (0.1 percent solution) on a coverglass and injected it with fluorescent dye, lucifer yellow[37)]. They stated that the injection can be confirmed visually by the fluorescence emitted by the dye. However, when this technique is used, due to the different positions of nucleus, cytoplasm, and vacuoles for each protoplast, the injection receiving site is not fixed when injected from one side. In addition, if the tonoplast is damaged during manipulation, the contents may leak into the cytoplasm causing cell death. On the other hand, Morikawa and Yamada[38)] modified and used a trapping pipette used for animal cells and developed a technique to fix the point of injection efficiently under a microscope. In injecting the DNA, which was visualized by the fluorescence emitted by berberine, into the protoplast of a cultured Euphorbia millii cell, they immobilized the protoplast by light suction of a semispheric trapping pipette. During this process, they succeeded in injection of the protoplast into the nucleus by rotating it with suctioning and releasing the protoplast with the tip of the trapping pipette so that the nucleus or the cytoplasm is positioned at the tip of the injection pipette. The injected protoplast was transfered to microculture by the pickup method, and the first cell division

was confirmed within one week.

The most important point in the microinjection technique is how to increase the injection efficiency. In animal cells, there is a case of treating as many as 7,000 cells per hour with a technique to pierce cells immersed in a DNA solution[39]. However, the current state in plants, even with improvement in techniques, seems to be about 10 cells per hour that can be used for culture[38]. Consequently, an improvement of culture techniques for DNA-injected protoplasts is the key to increasing the success rate, which remains as a problem for the future.

3.1.4 Electroporation technique

Although it has not been done in plants, Wong and Neumann[40] and Neumann, et al.[41] incubated mouse cells (TK^-) in a solution of DNA strands or ccc DNA containing the TK gene of HS virus, treated them with electric pulses of 8 kilovolts/centimeter, 5 microseconds, and obtained 500 transformants per 10^6 treated cells. Although the details of the genetic transduction mechanism are unknown, it is believed that the electric pulses make minute perforations in the cell membrane and promote the incorporation of DNA.

3.2 Chromosome engineering technique

3.2.1 Pollination with irradiated pollen

As this is not a transformation system on the somatic cell level, it is a technique that may be called "sexual transgenosis"[42]. This phenomenon was discovered during studies of "menthol pollen" used to overcome cross imcompatibilities present among species of the genus Nicotiana. The technique entails merely pollination following irradiation of the pollen of the pollen-donating parent. However, it demonstrates maternal heritability in which the maternal characteristics are expressed more strongly than in ordinary F1 and subsequent hybrids (Table 4). It has been confirmed that in the above process, part of the pollen parent gene can be transduced into a hybrid. When the irradiation dose is increased, although the tendency for maternal heritability becomes stronger, the characters of the pollen parent are always expressed. Consequently, it is believed to be applicable in replacing the backcrossing technique to transduce a small number of major mobile genes[43),44]. There are still many unknown points with regard to the mechanism of this phenomenon, but it is believed that the pollen chromosome fragments caused by irradiation are transmitted to the egg cell which subsequently

causing parthenogenesis of the egg cell and ripening of the seeds. In the process, the chromosome fragments of the pollen parent are incorporated into the maternal genome, which is subsequently stabilized and inherited. However, there is also a report[48] claiming that this technique is not a substitute for backcrossing.

Table 4 Transformation by pollination with irradiated pollen

(1)材 料 (特性)	(2)照射花粉供与親 (特性)	(3)照 射 線 量	(4)後代での形質発現	(9)文献
Nicotiana forgetiana	N. alata (6)(赤花など)	(7)γ線, 100krad	(8)赤花などが発現	42)
(5)(白花など) N. rustica, V27	N. rustica, V12	(12)X線, γ線, 10－20krad	(15)線量増加に伴い母親に似るが, V12の形質も発現	43)
(10)(体色緑など) N. rustica	(11)(体色黄など) N. tabacum	(8)γ線, 5－40krad	(16)雑種と区別できないものから母親に似るもの, および母親に似て稔性のあるものが出現	45)
(13)コムギ (cv. Chinese Spring)	(13)コムギ (cv. Hobbit)	(8)γ線, 2－5krad	(17)M1に異数性が出現, M1で稔性のあった2個体より得たM2では母親に似ていた	46)
(14)オオムギ (cv. Golden Promise)	(14)オオムギ (cv. Magunam)	(8)γ線, 0.5－2krad	(18)線量の増加に伴いM2は母親に似るが, 花粉親の特性もみられた	47)

key: 1) material (characteristics)
2) irradiated pollen donor parent (characteristics)
3) irradiation doses
4) characters expressed in progeny
5) (white flowers, etc.)
6) (red flowers, etc.)
7) gamma-ray
8) red flowers, etc. expressed
9) literature
10) (plant color of green, etc.)
11) (plant color of yellow, etc.)
12) x-ray, gamma-ray,...
13) wheat
14) barley
15) Resembles the maternal plant associated with increased doses, but V12 character was also expressed.
16) Plants that are indistinguishable from hybrids, those resembling the maternal plant, and those resembling the maternal plant and fertile were expressed.
17) Aneuploidy was expressed in M1, and M2 obtained from two fertile M1 individuals resembled the maternal plant.
18) M2 resembles the maternal plant associated with increased doses, but pollen mother characteristics were also expressed.

3.2.2 Cell technologies

The technique to transduce extracted metaphase chromosomes into the cells of other species for transformation was first

introduced in 1973 when the hamster ($HPRT^+$) chromosome was transduced into a mouse cell (hypoxanthine phosphoribosyl transferase$^-$; $HPRT^-$)[49]. Subsequently, studies with animals continued, and in 1980, an attempt began to extract metaphase chromosomes efficiently from plants[50].

Griesbach, et al.[51] extracted chromosomes from the root tips of seven plant species including lilies, broad bean, corn, etc. and pollen mother cells of lilies and Hemerocallis using the following technique. Root tips and pollen mother cells were incubated at room temperature in a solution (pH 5.7) containing 0.05 percent colchicine, 2 percent cellulysine [phonetic], 1 percent macelozyme [phonetic], 0.25 percent pectinase, 0.25 percent lozyme [phonetic], and 13 percent mannitol forming protoplasts of the cells while simultaneously pretreating. After incubating the root tips for 18 hours and the pollen mother cells for 30 minutes, they are suctioned and released with a Pasteur pipette and allowed to stand for another hour. The solution is filtered with gauze and centrifuged for 15 minutes at 200xg to collect the protoplasts. After washing twice with 20 [ml] capacity of 5 mM MES buffer solution, pH 6.0 (containing 13 percent mannitol) twice, protoplasts are suspended in a solution (pH 7.0) containing 15 mM HEPES, 1 mM EDTA, 15 mM DTT, 0.5 mM spermine, 80 mM KCl, 20 mM NaCl, 300 mM sucrose, and 500 mM hexylene glycol; and fragmented by passage through a 27 gauge needle 3-4 times. After centrifuging this solution for 15 minutes at 200xg and removing the cell residues, the metaphase chromosomes are collected by centrifuging for 10 minutes at 2,500xg. Although there were differences in yields with different materials, it was possible to recover 20-70 percent of the metaphase chromosomes using this technique. On the other hand, when extracting chromosomes from cultured cells, it is necessary to device a way to increase extraction efficiency by synchronizing the cell division cycle with drug treatment[52].

Metaphase chromosomes are transduced into protoplasts using the uptake method, liposome technique, or microinjection technique. With the uptake method, PEG is used to promote incorporation (Table 5). After treating Hemerocallis chromosomes obtained with the above-mentioned extraction method with DAPI, Malmberg and Griesbach[53] incubated the solution (pH 6.0) composed of 10^7 chromosomes/ml, 10^6 tobacco leaf mesophyll protoplasts/ml; 35 percent PEG 4,000, 2 percent mannitol and 12 mM $CaCl_2$ for 20 minutes and observed them under a fluorescence microscope. The results revealed that 1 percent of the treated protoplasts had incorporated chromosomes.

Table 5 Chromosome engineering in plant cells

(1) 材料	(2) 外来染色体の種類	(3) 処理条件 (4) 処理法	(5) 時間 (h)	(6) 温度(℃)	(7) 取込みの検定	(8) 文献
(9) コムギ, パセリ, トウモロコシ	(10) コムギ, パセリ	(11) PEG法	0.08 - 0.16	25	細胞学的観察	52)
(13) 培養細胞プロトプラスト	(15)	(11)				
(14) タバコ葉肉プロトプラスト	ユリ	PEG法	0.3	-	(16) 蛍光顕微鏡観察	54)
(14) タバコ葉肉プロトプラスト	*Hemerocallis*他 (18)	(11) PEG法	0.3	(17) 室温	(16) 蛍光顕微鏡観察	53)

key: 1) material
2) type of foreign chromosomes
3) conditions of treatment
4) method of treatment
5) time
6) temperature
7) incorporation test
8) literature
9) wheat, parsley, corn
10) wheat, parsley
11) PEG technique
12) cytological observation
13) cultured cell protoplast
14) tobacco leaf mesophyll protoplast
15) lily
16) fluorescence microscopy
17) room temperature
18) Hemerocallis and others

The liposome technique has not been tried in plants as yet. Mukherjee, et al.[55] extracted metaphase chromosomes from A9/HRBC2 human-mouse hybrid cells, encapsulated them in liposomes prepared from cholesterol and lecithin (this is called specifically lipochromosome), fused them with murine HGPRT-A9 cells using PEG 1,000 and obtained transformed cells that form colonies in HAT medium at a frequency of 10^{-5}. The frequency was reportedly 10 times greater than in the case without using liposomes.

Regarding the microinjection technique, Griesbach reported examples in 1984 at the "International Congress on Crop Genetic Manipulation" (Beijin) in which he tried the technique of removing vacuoles from protoplasts by centrifugation in a Percoll solution, fixing them with 0.6 percent agar, and injecting the chromosomes [into target cells] using several plant species. In order to increase the success rate, it is necessary to device ways besides removing the vacuoles such as concurrent use of a nuclease inhibitor and increasing the chance of injection into M phase cells by synchronizing the recipient cell divisions. He stated that 58 percent of the protoplasts treated in this manner began dividing. Recently, testing is reportedly under way for petunia plants redifferentiated from protoplasts in which a gene group controlling drought tolerance was transduced using this technique[56].

As chromosome engineering techniques are improved, our interest

is directed toward the feasibility of transduction of a specific chromosome alone as discussed in section 2. However, in order to achieve this, it is necessary to establish an isolation technique pertinent to each chromosomal karyotype as well as construct genetic maps of higher plants in further detail covering extensive species. Malmberg and Griesbach[53] attempted to fractionate metaphase chromosomes using continuous sucrose density gradient centrifugation. However, in an analysis using the rRNA gene (in SAT-chromosome) as the marker, although there was a peak, distribution was disperse and fractionation was inadequate. Further improvement is necessary on these points. Regarding genetic map construction, progress in basic research is desirable in genetic analysis of various characters including physiological and biochemical characters as well as the analysis of linkage groups.

4. Potential for production of useful substances (Conclusion)

As described above, progress in DNA and chromosome engineering in animal cells greatly stimulated and promoted research in plant cells. Although one cannot state that these techniques will be immediately useful in breeding, it can at least be stated that they opened a direction for new breeding techniques. However, on the other side, they resulted in demonstrating how deficient our knowledge is regarding eukaryotic genomes and genes in making full use of these techniques. This problem is also true in the case of genetic control of secondary metabolites produced through a long biosynthetic pathway. Examples[57] showing that selection of a mutant strain for abnormal accumulation of primary metabolites in cells does not always lead to increased production of useful substances indicate that selection is not always made in the desired direction regarding the genotype of the secondary metabolic system. It is believed that either the genetic components controlling the secondary metabolic system do not correspond to the increased production or the system as a whole is inhibitorily regulated[58], but the details are unknown.

As has been stated, DNA and chromosome engineering have the special feature of permitting transduction of a large amount of genetic information at once. Consequently, even though details of genomes and genes are not yet known, transformation itself can be attempted at any time. Then, the fast route to accumulate knowledge in this field and to gain an applicable prospect is to continue with basic research on genetic analysis or regulatory mechanisms of secondary metabolites on one hand, while actively promoting research to extract DNA or chromosomes from strains showing a high production of useful substances and transducing them into low-producing but fast propagating cells or cells of other species that do not produce that substance.

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BIOTECHNOLOGY

NEW TECHNIQUES IN INSECTICIDES, HERBICIDES DISCUSSED

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[Article by Prof Shoichi Matsunaka, Kobe University Agriculture Faculty]

[Text] A general explanation is given of the contributions the new techniques of biotechnology have made and may yet make to protection of crops, particularly in the fields of eliminating damage from noxious insects and weeds. It deals specifically with the breeding of plants resistant to blight and herbicides, and with productivity improvement of agricultural chemicals produced by or consisting of microorganisms.

1. Introduction

The topic in the request I received from the editors was "biotechnology for new agriculture and agricultural chemicals." But on reconsideration of the special edition as a whole, it was decided to make it a general explanation of the current status and possibilities of biotechnology in the field of crop protection.

Agricultural chemical scientists of the Japan Agricultural Chemical Society planned and executed a special edition of their journal, with the title "Biotechnology and Crop Protection." It put forward five general surveys: a) its status and future[1], (b) biotechnology and breeding resistant crops[2], (c) genetic manipulation and microbial degradation of environmental pollutants[3], (d) genetic manipulation and agricultural chemicals produced by microorganisms[4], (e) application of biotechnology to microbial agricultural chemicals[5]. Backed up by those general surveys, the present paper attempts to present an overall view, with emphasis on my own specialty of resistance to herbicides.

A movement called "biopesticides," which uses such things as pheromones, has emerged in the context of crop protection, but I will limit the range to which biotechnology in the narrow sense can contribute (aside from the production of virus-free starts by means of shoot apex cultivation) to giving crops various kinds of resistance and to agricultural chemicals of microbial origin.

2. Providing (breeding) resistance to an adverse environment:

Crops once carried out their life activities while receiving a supply of nutrients and moisture in a medium such as soil, and using all the solar energy they needed. However, a number of negative factors now exist in the environment. These include blight, noxious insects and weeds, agricultural chemicals intended to eliminate those three (chemical damage), heavy metals and salts in the soil, insufficient moisture (drought) and so on. Removal or control of these negative factors is the essence of crop protection. In addition to direct removal of these negative factors, providing crops with resistance against an adverse environment is effective as a means of crop protection. Providing resistance to blight and noxious insects has long been a major objective of crop breeding, and it will continue to be. It is hoped,however, that the new biotechnology can be put to use in the introduction or selection of resistant characteristics.

2.1 Introduction of resistant characteristics: Heretofore the breeding of crops with resistance to blight, noxious insects, etc., has been a matter of introducing characteristics by cross-breeding with resistant members of the same or closely related species. Now four new methods are conceivable: change of characteristics using recombinant DNA, introduction of characteristics with a virus as a vector, zygotic transmission using transmittable plasmids, and cell fusion.

2.1.1 Introduction of characteristics using vectors: Hess once reported producing a pink petunia by putting DNA from a red petunia into a white one; now it has become necessary to find suitable vectors for introduction or manifestation of the desired genes (DNA) into other plants. Such things as single strand DNA viruses and cauliflower mosaic virus (double strand DNA) or 90~150 megadalton Ti plasmids of Agrobacterium tumefaciens, which produces crown gall in dicotyledonous plants, can be used as vectors.

As an example of the introduction of a target gene into higher plants using Ti plasmids as the vector, it is known that the ability to break down kanamycin, the ability to synthesize various enzymes, and resistance to methotrexate have been transferred, primarily to tobacco[6], but these were all cases of microbial genes being linked to the nopalin synthesizing enzyme of the Ti plasmid. Murai[7] introduced Ti plasmids combined with phaseolin protein synthesizing kidney bean genes into sunflowers.

These experiments are important as models for methods to introduce various genes for resistance into higher plants, the issue under consideration here, but unfortunately it is not yet possible to freely introduce a desired gene into a target plant.

There is recent news that a Calgene Company group is obtaining genes for resistance to the herbicide glyphosate (a gene which synthesizes a glyphosate resistance enzyme, which has selin in place of prolin in the 101st position of the enzyme which synthesizes 5-enol pyruvyl shikimate 3-phosphate) from Salmonella typhimurium and transferring it to plants.

Skillfully using Ti plasmids and pPM 54 plasmids from colon bacilli, they succeeded in transferring that gene to turnip gall.[9] As in table 1, the EPSP synthesis enzymes from turnips with changed characteristics reduced the retardant effect of glyphosate more than those from ordinary turnips; where the latter enzyme had no reaction to antiserum against bacteria enzymes, 58 percent of the former were affected. From that it was concluded that about 50 percent of the enzymes from turnips with changed characteristics were the same as enzymes from resistant bacteria. Through the change of characteristics they succeeded in making the bacteria's enzyme for resistance show up in plants, but further experiments are needed on whether or not the plants are resistant to glyphosate. If those experiments succeed, it will be possible to use glyphosate, a nonselective herbicide with few problems in terms of environmental pollution and toxicity to animals, as a selective herbicide for these plants.

Table 1. Glyphosate resistance and immunological characteristics of EPSP* synthesis enzyme transferred to plants from glyphosate-resistant bacteria

	(a) EPSP 合成酵素活性（対照の%）		
(b) 阻害剤 (c)	抵抗性細菌の酵素 (d)	普通のカブのゴールの酵素 (e)	形質変換したカブのゴールの酵素
(f) なし	100	100	100
(g) グリホセート			
100 μM	35	8	29
500 μM	9	2	9
(h) 抗血清**	1	115	42

(i) * 5-エノールピルビルシキミ酸-3-リン酸
(j) ** 細菌の EPSP 合成酵素に対する抗血清

Key:
(a) EPSP synthesis activity (relative percentage)
(b) Retardant
(c) Resistant bacteria enzyme
(d) Ordinary turnip gall enzyme
(e) Changed characteristic turnip gall enzyme
(f) None
(g) Glyphosate
(h) Antiserum**
(i) * 5-enol pyruvilshikimate-3-phosphate
(j) ** Antiserum against EPSP synthesis enzyme in bacteria

2.1.2 Introduction of characteristics by cell fusion: A naked cell enclosed just in the cell membrane, with the cell wall removed by treatment with pectinase and cellulase, is called a protoplast. Cell fusion, in which protoplasts of different kinds are joined to each other by means of stimulation with a chemical such as polyethylene glycol (PEG)[10] or with electrical stimulation,[11] has received attention as another method of introducing desired characteristics. The "pomato" created by fusion of potato

and tomato cells is quite famous. It is particularly useful in cases where creation of recombinant DNA is difficult or in cases where the vector to carry the recombinant DNA has not been developed, such as for transmission of genes to chlorophyll. But because unnecessary or undesirable characteristics can be introduced by fusion, along with the desired resistance, what are called "Cybrids" have also been produced by destroying one nucleus and just fusing the cytoplasm.

Beversdorf et al.[12] crossed and recrossed the weed Brassica campestris, which is resistant to the herbicide atrazine, with B. napus, a type of Argentine rape, and succeeded in introducing its resistance into the rape. Pelletier took the nucleus of B. napus and produced a cell hybrid with the mitochondria of Raphanus sativus and the chlorophyll of B. campestris. In this hybrid he discovered the nuclear genes of B. napus, the male sterility of R. sativus cytoplasm and the atrazine resistance of B. campestris.

In addition, Gressel et al.[13] tried using cell fusion to introduce the atrazine resistance of black nightshade (Solanum nigrum) into the Irish potato (S. tuberosum). They succeeded in isolating protoplasts from both plants, fusing them, callusing and redifferentiating the result. This redifferentiated organism had 96 chromosomes, the total of the two plants (72 plus 24), and it was resistant to atrazine, but the all-important tubers did not form.

The introduction of the characteristic of resistance can be considered a direct method which avoids reliance on chance, but there seem to be many difficult problems which must be overcome before we can use it freely.

2.2 Selection of resistant cells: the selection of resistant organisms is the first step of an important means of breeding crops resistant to blight and noxious insects. The new biotechnology has brought the level of the resistant bodies down to the cell level. It is thought that the larger the number of objects involved, the greater the chance of success for the selection operation. Natural variation operations can be augmented with the purpose of broadening the range of the variation, but shifting from selection at the organism level to selection at the cell level makes it possible to deal with larger numbers, and raises the chance of success. And at the cell level, it is possible to add in such things as the cell fusion techniques mentioned above (when cell fusion is used, the determination of whether the object of the fusion was achieved is of course made using various selection methods).

The selection operation for various resistant stocks is, generally speaking, to apply the stress or pressure which is the object of the resistance to cultures of cells of the crop in question, select out only those which survive the stress, cause them to multiply, redifferentiate them, and double the haploid number with colchicine, for example, to obtain resistant organisms. For cell cultures it is possible to use single cells or a clump of cells in a liquid culture medium, a callused clump on a solid medium, a group of protoplasts, etc.

In cases of plant disease, the cause of the disease is often controlled by factors other than toxins from the pathogen, but it is also common that the

production and action of toxins is the primary factor. In such cases, it is possible to apply the toxin as pressure, and select the cells or cell clumps which survive. It should be possible to use the same process when the cause of the disease is not a toxin, but a particular enzyme.

Carlson [14] prepared protoplasts from a variety of tobacco sensitive to wildfire (Pseudomonas tobaci), selected resistant cells in the presence of a substance similar to the toxin from the pathogen (methonine sulfoxymin), and succeeded in regenerating resistant plants from those cells.

The major factor in increased yields of corn in America is hybrid vigor resulting from hybridization, but one strain with cytoplasmic male sterility used for seed production has been greatly damaged by the appearence of race T of Dochliobolus miyabeanus, to which that strain is particularly sensitive. By applying race T toxin to a callus culture of corn sensitive to it, it was possible to obtain resistant plants from cells which occured through natural mutation.[15] The gene for sensitivity in corn is present in the mitochondria; when sensitive and resistant mitochondria were compared, differences in the DNA configuration were discovered.[16]

On the other hand, Shepard et al., using potato leaf protoplasts, discovered some which showed resistance to toxins from leaf blight (Phytophthora infestan) and early blight.[17]

Heinz obtained sugarcane resistant to cane spot and its toxin, helminthosporin, by callus and suspension cultures, and reported that some of these were also resistant to mosaic virus and downy mildew.[18]

In Japan, Kurosawa et al. used toxins from tobacco wildfire and Gymnosporangium japonica for selection in a callus culture from tobacco protoplasts, and obtained organisms resistant to each.[19]

In addition to the above, the same methods have been used successfully with potato wilt[20] and rapeseed root rot.[21]

The greatest development in this field is seen in the production of crops resistant to herbicides.[22] In each case, resistant plants have been obtained by adding the herbicide in question to protoplast, cell or callus cultures, then isolating, multiplying and regenerating the survivors.

An overwhelming number of successful cases have dealt with tobacco. This reflects the fact that various cell engineering methods have shown marked progress with tobacco. If the the basic techniques of cell engineering for other crops can reach the levels accomplished with tobacco, it will be quite possible to produce herbicide-resistant plants.

If I may mention a few particularly noteworthy successes, there are the examples of fenmedifam and bentazon, both herbicides of the photosynthesis obstructor type. There is no dependence on normal photosynthesis in the callus culture stage, so the fact that resistance to the two herbicides was present in selection at that stage did not guarantee resistance once a plant hd been redifferentiated, turned green and begun to rely on photosynthesis.

Consequently Radin and Carlson[23] exposed haploid tobacco leaves to X-ray irradiation to cause mutations, allowed further growth, then applied herbicide just to the new leaf buds. Assuming the "green islands" among the white leaves (to which herbicide had been applied), which grew with the assistance of photosynthesis in the portion of the plant to which no herbicide was applied, to be resistant tissues, they grew callus cultures from these parts and thus regenerated resistant plants. They were 21 percent resistant to bentazon and 13 percent resistant to femedifam. After obtaining diploid forms with colchicine, the two were cross-bred, and a recessive gene for resistance was indicated.

Sulfonyl urea herbicides such as chlorosulfuron and sulfometulon methyl kill vegetation at low concentrations of 1 to 2 mg/m^2 (10 to 20 g/ha). But because they block the synthesis of the essential amino acids valine and isoleucine, their animal toxicity is actually quite low. They are powerful retardants of acetolactate synthesis enzyme (ALS), the key enzyme in synthesis of branched amino acids, and their I_{50} is on the order of 1 nM. The Du Pont Research Group, which developed these herbicides, first treated haploid tobacco calluses with 1 mM ethyl nitrosol urea for three weeks to cause mutation, added 2 ppb (about 5.6 nM) of the two herbicides to select the survivors, and multiplied them. They were then subjected to two more three-week passages, regenerated and multiplied. The regenerated plants were resistant to the two herbicides. The genetic mode indicated a single dominant or semidominant gene.[24] The resistant tobacco plants, when treated with 100 ppm chlorosufuron showed the same growth as untreated plants, demonstrating practicality.

The ALS of the resistant tobacco obtained in this way indicated a decline in sensitivity to the two herbicides. And it has been reported that, in an experiment using microorganisms, in addition to the ALS of stocks resistant to sulfonyl urea herbicides showing resistance to these herbicides, the enzyme for resistance underwent a change of one proline to serine among the amino acids making up the protein structure of the sensitive enzyme.[25]

According to a recent personal letter from Dr Shaner of the ACC Company, they have succeeded in producing a new variety of corn which resists imidazolynon herbicides such as AC243,997 (imazapyr, brand name Arsenal) developed by that company. This herbicide is an ALS retardant like the sulfonyl urea herbicides mentioned above, but is not as strong. According to the letter, P.C. Anderson and others of Molecular Genetic Company added (to yield a concentration of 0.03 ppm) imazaquin, an herbicide similar to imazapyr, to a callus culture started from an isolated corn germ, repeated this process for seven generations (raising the concentration to 0.1 ppm in the 5th generation) and succeeded in obtaining a resistant callus from which regeneration was possible. When pollen from corn obtained in this way was crossed with a separate strain of corn, the resultant seed showed 41 percent resistance to imazaquin.

These results indicate that this resistance is developed in the whole plant, not just the callus, that it is an inherited characteristic, and that it is probably controlled by a single dominant gene. The ALS extracted from the resistant callus shows 1000 times the normal resistance to imazapyr and

imazaquin; in this case too it is thought that a change in the sensitivity of ALS to the herbicide is the source of the resistance. It should be noted that this result is a success, not with tobacco, but with corn, which is both harder to redifferentiate and a more important crop.

In addition to these results, similar methods have produced paraquat-resistant tomatoes[26] and tobacco resistant to atrazine or to aminotriazole (ATA).[27]

The Du Pont Company plans to sell "crop packages" which include seed for these herbicide-resistant crops; there is a good deal of practical activity in this field.

3. Microbial crop protection

As seen in the commercial mass production of human insulin by colon bacilli, the world of microorganisms is without question the productive center of biotechnology. Consequently, biotechnology can also be used to advantage where microbes can make a contribution in relation to crop protection. We can consider both the case of putting the microbes themselves to use, and the case of using products of the microbes.

3.1 Using microorganisms themselves: Frost damage to crops occurs because ice crystals form around bacteria on the leaf surfaces. There have consequently been attempts to prevent frost damage using intermicrobial rivalry by isolating, multiplying and propagating similar bacteria which are very fertile but lack the capacity for forming ice crystals.[28] However, cases where the microbes themselves have been used have been primarily matters of controlling noxious insects or disease. When the use of microbes for the control of noxious insects is considered, what is called biological control (use of natural enemies) comes to mind. The natural enemies in these cases are often insects or acarids, but microorganisms are also used. The use of natural enemies can be divided into xcontinuing use and temporary deployment, and the latter can be further divided into spot application and mass distribution methods. In the case of mass distribution, the natural enemy organisms can be thought of as living pesticides (Yasumatsu has said living pesticides are spread or released each season when the noxious insects emerge, and their effect does not extend beyond one year[29]). Cases of the use of molds as biological weed killers have also been reported.

3.1.1 Use against noxious insects: Those things other than viruses used as living pesticides to control noxious insects are shown in table 2.[5),30] Viruses will be discussed hereafter.

Table 2: Microorganisms used as biological pesticides[5),25)*]

		(c) 微生物	代表的標的害虫	(d) 国別
(a)	糸状菌	*Aschersonia placenta*	ミカンコナジラミ	(e) ソ連
		Aschersonia aleyrodis	ミカンコナジラミ	(f) 日本
		Beauveria bassiana (Boverin)	コロラドハムシ	(g) ソ連
		Beauveria tenella	コフキコガネ	(h) フランス
		(= *brongniartii*)	ドウガネブイブイ	(i) 日本
		Hirsutella thompsonii (Mycar)	ミカンサビダニ	(j) アメリカ
		Metarhizium anisopliae (Conbio)	アワフキムシ	(k) ブラジル
		Nomuraea rileyi	オオタバコガ	(l) アメリカ
		Verticillium lecanii (Vertalec tated Lyle)	ヒラタカタカイガラムシ	(m) チェコ
(b)	細菌	*Bacillus lentimorbus* (Milky spore powder)		
		Bacillus moritai	イエバエ	(n)
		Bacillus popilliae (Doom)	マメコガネ	(o) アメリカ
		Bacillus sphaericus	カ	(p)
		Bacillus thuringiensis		
		subsp. *kurstaki* (Dipel 等)	鱗翅目昆虫	(q) 広範囲
		subsp. *israelensis* (Tekner Bactimos)	双翅目昆虫	(r) 〃
		subsp. *aizawai* (Certan)	Wax moth	(s) アメリカ

(t)* ウイルスを除く。（　）内は製品の商品名

Key:
(a) Mycetes
(b) Bacilli
(c) Microorganism
(d) Representative pest, country
(e) Tangerine louse, USSR
(f) Tangerine louse, Japan
(g) Colorado beetle, USSR
(h) June beetle, France
(i) Dogane buibui, Japan
(j) Orange rust mite, U.S.
(k) Spittlebug, Brazil
(l) Tobacco fly, U.S.
(m) Hiratakata scale, Czechoslovakia
(n) Housefly
(o) Japanese beetle, U.S.
(p) Mosquito
(q) Lepidopterous insects, widespread
(r) Dipterous insects, widespread
(s) Wax moth, U.S.
(t) * Excluding viruses. (Brand names in parentheses)

In Japan it is necessary to give continual consideration to the effects of mycetes and bacilli on silk cacoons and on the existence of sericulture. It is reported that the mycete B. bassiana exists in a form which is a strong pathogen against silkworms and in a form which is weak against silkworms but strong against leafhoppers (Nephotettix bipunctatus cincticeps).[31)] A difference is also reported in resistance to high temperatures. There are

examples of success in the directions of protoplast preparation, cell fusion and genetic recombination in the breeding of mycetes (benlate resistance in Paecilomyces fumoso-roseus[32]), but the accumulation of fundamental methods is still necessary.

Among bacilli, B. thuringiensis is the most famous and most studied in Japan.[5] It produces four toxins: delta-endotoxin, alpha-exotoxin, beta-exotoxin and gamma-exotoxin. The pesticidal effect is greatest in delta-endotoxin, which forms within the bacillus during spore formation, and beta-exotoxin, a heat-resistant substance with a low molecular number that is excreted into the culture fluid. The former is a crystalline protein made up of subunits with a molecular weight of 1.45×10^5; its active fragment is a polypeptide consisting of 300 to 500 amino acids. When taken orally, it is broken down by protease in the insects' digestive fluids and changes to an active toxin. Moreover, in some subspecies there are two or more varieties of the crystalline toxin.

The structure of the low molecular number beta-exotoxin has been determined by Farkas et al.[33] It is an adenosine derivative that blocks RNA synthesis; it is not used in Europe and the U.S. because of toxic aspects for mammals, but it is used in the Soviet Union.

One biotechnologically interesting point in this field is the discovery that delta-endotoxin is controlled by a B. thuringiensis plasmid gene. Whitely et al. give a detailed presentation of research on the cloning of this gene.[34] They took plasmids from subsp. kurstaki HDI and, after genetic manipulation, succeeded in introducing them into colon bacillus HB 101 and synthesizing delta-endotoxin protein. In addition, research on genetic engineering synthesis of delta-endotoxin, using colon bacilli or hay bacilli has been conducted successfully in a number of places, including the Suntory Group in Japan. It appears that production of the toxin protein and formation of the crystalling substance are governed by different genes; a mixed culture has been effective in allowing crystallization.

B. moritai has been isolated from Japanese soil, and is authorized as a biological pesticide. Its spores are effective; if mixed in cattle feed and fed to cattle, the spores are excreted with fecal material. Fly larvae that hatch from eggs laid on these feces eat the spores, which then germinate, multiply and kill the larvae. In connection with biotechnology, there have been experiments to transfer genes of a mutant strain of B. thuringiensis to B. moritai, and experiments in selection of strongly pathogenic bacilli.[5]

B. popilliae has been commercialized in the U.S. under the brand name "Doom." Although it is effective against Japanese beetles, it is not pathogenic for the dogane buibui and the hime beetle (Anomala rufocuprea Motshulsky) which are important in Japan. A biotechnological explanation of this difference might expand the applications of this bacillus.

The virus which is used the most is Baculovirus, a nuclear polyangular virus (NPV). NPV is double-strand DNA with a molecular weight of 70 to 130 megadaltons. In Japan it has been studied to the point of practical application for eradication of hasumon yoto.[36] Moreover, the kokakumon

hamaki granular virus (GV) in tea and apples is also included in the Biotechnology Use Promotion Project of the Ministry of Agriculture, Forestry and Fisheries. The eggar (Dendrolimus spectabilis--not included among pine weevils) CPV, which belongs to Reovirus, has been registered as a pesticide with the name "Matsukemin."

Viruses consist of nucleic acid and are well-suited as subjects of biotechnology research. Research has been conducted on genetic manipulation of Autographa californica NPV, using DNA genome gene mapping and recombinant DNA techniques.[5] Mass production of viruses involves technological problems, but research has been conducted using cell cultures.[370]

3.1.2 Use against blight: To make practical use of microorganisms for the purpose of preventing blight, it is first necessary to provide weak viruses. Using the phenomenon that crops treated in advance with weak viruses having reduced pathogenicity do not develop diseases when later exposed to strong viruses, weak virus $L_{11}A$ obtained by treatment of tomato stalks infected with tomato TMV for two weeks at 35°C was put to practical use in hothouse tomato culture.[38] Cucumber mosaic virus, citrus tristeza virus and pepper mosaic virus have been put to use in the MAFF Biotechnology Use Promotion Project mentioned above. Much remains for biotechnological consideration, including why weak viruses have this interference effect, as well as methods for creating stable weak viruses and measures to keep them from growing stronger.

As stated above, plasmids have been discovered in mycetes and used for microbial pesticides, but plasmids have also been prepared from vegetable pathogen mycetes. In the case of Rhizoctonia solani, the plasmids are introduced into wild stocks by the polyethylene glycol method, and mycelium fusion lowers the pathogenicity of the wild stock; crop diseases can be controlled with both stocks growing together.

Use of microorganisms to combat sweet potato vine-snap, nonpathogenic fusarium and particilium are also included in the MAFF project. It will be necessary to put greater effort into a proper understanding of the interference effect among microorganisms, with precise regulation of experimental conditions and sure identification of the microbes being used.

3.2 Using products of microorganisms: If the toxins of the previously mentioned B. thuringiensis were mass produced by colon bacilli and put to use, they would fall in this classification, but I will concentrate here on production of materials for crop protection, primarily agricultural antibiotics.

Microbial products other than antibiotics have been in use for some time; these are broadly referred to as microbial angricultural medicines. Almost all are produced by actinomyces, and so the development of biotechnology in this field has paralleled that of cell fusion and genetic manipulation of actinomyces.

Since the process of protoplast preparation and regeneration were developed for actinomyces about ten years ago,[39] genetic cross-breeding between stocks, species and genuses has become possible using cell fusion. Ogawa et al., with

the goal of increasing the capacity of Streptomyces hygroscopicus to produce the herbicide bialaphos, prepared protoplasts from a stock with high productivity and from a stock with low capability to break down bialaphos; they fused them using PEG, and produced a fused product with almost no capability for decomposition and with higher productivity than either of the parent stocks.[40]

Not surprisingly, plasmids are used as vectors in actinomyces gene manipulation; the plasmids pIJ 61 and pIJ 702 are useful. Recently Okanishi et al., using the latter to place DNA from S. kasugaensis stock M518 into stock 1121, obtained a stock with ten times the capacity of either parent stock for production of kasugamycin (a germicide used for rice blast).[1] The Yano paper in the series mentioned at the beginning[4] has details on genetic manipulation for production of microbial agricultural medicines.

Aside from microorganisms, plant cell cultures have also been used for production of biochemical components, plant pigments and so on. From the perspective of crop protection, A substance that is effective against tobacco mosaic virus has been obtained from pokeweed (Phytolacca americana) cell culture broth,[41] and a substance with anti-TMV activity has been obtained from cornflower (Agrostemma githago) cell culture.[42]

4. Conclusion

I have tried to state here an overview of biotechnology in the field of crop protection. The underlying point is that although fairly good results have been obtained, we seem to be a step short of full practicality. I sense an impatience with the fact that basic techniques such as introduction of DNA, cell fusion and redifferentiation from callus have succeeded only with a very limited number of plants. It is necessary to give deep thought to the importance of the role to be played by steady perfection of basic techniques. Thus we should carefully avoid the hasty evaluation of research results.

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ENERGY

PRESENT LEVEL OF a-Si SOLAR CELL TECHNOLOGY

Tokyo NIKKO MATERIALS in Japanese Aug 85 pp 16-20

[Article by Sakae Hayami: "Challenge to a Bigger Area--Amorphous Solar Cell"]

[Text] The market for solar cells which utilize inexhaustible solar light by directly converting it to electricity is rapidly expanding. Three kinds of solar cells--amorphous, polycrystal, and single crystal silicon cells--have already been produced industrially. Of these three types of cells, the amorphous (noncrystal) solar cells, which provide the possibility of great cost reduction, have accounted for more than 50 percent of production. The amorphous solar cell manufacturers are engaged in fierce development competition for higher performance, lower cost, and a larger market area.

Produced Industrially in 5 Years After Announcement of Theory

The announcement made by (W.E. Spare) and his group at Dundee University in the United Kingdom that P-N junction is also possible in amorphous silicon (a-Si) by using glow discharge, resulted in confirming that a-Si functions as a semiconductor. Only 5 years later the production of a-Si solar cells for civilian use began. The basic research work on a-Si solar cells for power generation commenced in 1980 under the government's Sunshine Project and an intrinsic conversion efficiency of 8 percent (10 cm^2) was achieved in 3 years. This research demonstrated that it is feasible to commercialize a-Si solar cells as a power source. So, the research to put them to practical use was started in 1983 led by the New Energy Development Organization (NEDO). The companies participating in this project are Sanyo Electric Co., Ltd., Fuji Electric Co., Ltd., Teijin Ltd., Komatsu Electronic Metals Co., Ltd., Mitsubishi Electric Corp., Kyocera Corp., Hitachi Ltd., and Sumitumo Electric Industries, Ltd., eight in all. In addition, Sharp Corp., Taiyo Yuden Co., Ltd., Kanegafuchi Chemical Industry Co., Ltd., TDK Corp., Showa Shell Sekiyu K.K., and Toa Nenryo Kogyo K.K. are carrying out the production and research of a-Si solar cells.

The characteristics of the a-Si solar cell are: 1) a solar cell can be formed with a thin film; 2) it is fit for mass production; 3) the production cost is low; and 4) it can be formed on a wafer of various kinds (glass, stainless steel, and film).

Sanyo Electric Co., Ltd.
11.5 Percent Conversion Efficiency Attained

At the first international conference on solar power generation held in Kobe in November 1984, Sanyo announced that its small, 1 cm^2 amorphous silicon (a-Si) solar cell had achieved a photoelectric conversion efficiency of 11.5 percent, the highest ever in the world. This comes to 9 percent in terms of the photoelectric conversion efficiency of a large area type (100 cm^2) for power generation and moved forward the commercialization of a large area type solar cell targeting a 10 percent level.

The technological development that succeeded in attaining the 11.5 percent can be summed up in the following two points: 1) The structure of the a-Si solar cell comes first. The structure of the existing a-Si cell is of a multilayer type comprising a glass wafer, transparent electroconductive film, a-SiC (silicon carbide) film, a-Si film, and an aluminum layer. While the transparent electroconductive film was made refractive by making it uneven, the efficiency to reflect the solar light with long waves was improved by replacing the aluminum layer with that of silver. With this new structure, the solar light which once entered the cell, finds it difficult to go out, being arrested by the indented transparent electroconductive film, so the light can be effectively utilized. The solar light with long waves, which a-Si can hardly absorb, can be gradually converted into electric energy through a number of repeated reflections inside the cell, and using silver in place of the aluminum layer. 2) The development of the technology to eliminate impure substances from the electrically neutral layers of the a-Si film resulted in improving the electric characteristics.

Sanyo Electric's central research institute defines the development of the above-mentioned solar cell as a milepost on the road toward an a-Si solar cell for power generation, saying, "The grounds have been established for achieving the goal of 10 percent conversion efficiency even for large area-type amorphous silicon solar cells."

Toward Mass Production System--10 Million Units Monthly (in terms of an electronic calculator)

In September 1980 Sanyo Electric became the first company in the world to sell desk-type electronic calculators with built-in a-Si solar cells, which are now quite popular.

The Sumoto Plant located in Awaji Island (Sumoto City, Hyogo Prefecture), the hometown for the three brothers of Iue family, the founders of Sanyo Electric, is the largest factory in the world mass-producing a-Si solar cells (brand name: (Amolton)). The new factory building constructed there in April 1985 has expanded the production capacity of solar cells from 5 million units (in terms of an electronic calculator) to 10 million units monthly.

Sanyo Electric's solar cell industrialization started in May 1980 at the pilot plant of Yodogawa with the monthly production of 5,000 units (in terms of an

List of Conversion Efficiencies of a-Si Solar Cells

(Source: NEDO)

Developer/ cell type	Kind of wafer	Intrinsic conversion efficiency (percent)	Area cm^2	Voc V	Jsc mA/cm^2	FF	Remarks
Mitsubishi--multilayer	Stainless steel	8.6	100	2.22	6.41	0.604	
Fuji--single layer	Glass	11.1	1.0	0.864	17.6	0.730	
" "	"	8.0	100	8.45	14.4	0.654	20 partitions
" "	"	7.3	600	45.68	13.5	0.57	54 partitions
Sanyo "	"	11.5	1.0	0.869	18.9	0.70	
" "	"	8.3	100	12.0	15.7	0.617	14 partitions
" "	"	8.68	0.04	0.835	15.7	0.662	5A°/sec
Kyocera "	Ceramics	8.52	1.0	0.87	14.0	0.70	
" "	"	7.31	100	4.35	19.4	0.622	5 partitions
Teijin "	Film	8.9	1.0	0.89	16.2	0.62	Sheet type
" "	"	7.4	100	0.89	13.8	0.60	"
" "	"	8.0	1.0	0.90	13.24	0.664	Roll type

electronic calculator) and in February 1982 the first mass production line (1 million units monthly) started operation at the Sumoto Plant. During these periods electronic calculators, wristwatches, and TV sets, all fitted with solar cells, were marketed in quick succession as a means to test applications.

Mass production and the improvement of photoelectric conversion efficiency are the tasks for Sanyo Electric, the top manufacturer of amorphous silicon solar cells.

1976--1.0 percent (1x1-mm)
1978--2.0 percent (2x2-mm)
1979--3.2 percent (35-mm x 35-mm)
1980--5.6 percent (a small area)
1981--7.9 percent (a small area), 5.6 percent (100 x 100-mm)
1982--8.82 percent (a small area), 7.21 percent (100 x 100-mm)
1983--10.0 percent (a small area), 7.8 percent (100 x 100-mm)
1984--11.5 percent (a small area)

The technology which provided an improved conversion efficiency to the a-Si solar cell is attributable to the development of an integrated type a-Si solar cell [as published].

The integrated type a-Si solar cell is so designed as to obtain high voltage by cascade-connecting a number of cells on one wafer, taking advantage of the characteristic of plasma reaction which forms an a-Si layer. Each cell formed on one insulated wafer is connected in series by way of proper patterns to the neighboring cell through the transparent electrode film and the electrode on the reverse side. The integrated type a-Si solar cell is fit for mass production since it can be pattern-molded like the production process of IC's and dry process can also be applied for formation. At present the (Amolton) Tile, which is the integrated type a-Si solar cell directly formed on a glass tile, is being commercialized in addition to the applied products such as clocks, radio receivers, TV sets, and tape recorders.

Sanyo Electric decided in May of this year to construct in Matsushige industrial park an amorphous solar cell production plant, which is expected to start operation in 1988.

Fuji Electric Co., Ltd.
More Spacious Cells Eyed

Fuji Electric, a mass-producer of a-Si solar cells rivaling Sanyo Electric, has raised its production capacity to 4 million units monthly by increasing production lines at its Matsumoto Plant, Matsumoto City, Nagano Prefecture.

Fuji Electric and Fuji Electric General Research Institute succeeded in developing an a-Si solar cell achieving an 11.1-percent conversion efficiency per 1 cm^2 in September 1984. The first reason for the success was that the p-layer was made transparent by inserting carbon to take in more light. The

second reason was that in properly arranging the bond length with the i-layer which generates electricity, electrons and holes were prevented from diminishing by gradually reducing the amount of carbon. Furthermore, the film quality of the i-layer itself was improved. All these factors contributed in attaining the conversion efficiency of 11.1 percent.

Having developed in 1978 in the initial stage of research and development activities of the spacious 49 cm^2 (7x7-cm) a-Si solar cell, Fuji Electric is now pushing forward the development project in stages from 100-cm^2 to 600-cm^2 (20 x 30-cm), 1,200-cm^2 (30 x 40-cm) and 4,800-cm^2 (40 x 120-cm) cells. Presently, a 6.3 percent conversion efficiency is achieved with a practical, large 600-cm^2 cell.

To cope with the demand for the expansion of the cell area, Fuji Electric has developed an IVE (vertical electrode multilayer distribution type)-shaped a-Si film forming device, which increases the throughput by expanding the total area of the wafer during the film forming process. The special features of this device are: 1) While wafers are arranged horizontally in the existing devices, this device arranges wafers and electrodes vertically to avoid the development of defects such as pinholes; 2) one reaction chamber has a number of plasma discharge zones, so the throughput can be raised by using a broader wafer and increasing its packed density.

Various Shapes Available by Fine Finishing Technology

Having established fine processing technology to put a-Si solar cells into various shapes, Fuji Electric has commenced their mass production. This permits it to produce solar cells in various shapes such as square and octagonal in response to a variety of watch designs. Fuji Electric is the first maker to mass-produce a-Si solar cells in many different shapes and this was made possible by two technologies.

One is fine processing technology. Since each cell for wristwatches was small in size as compared to that for electronic calculators, such cells were made finer, and at the same time mass production lines were installed introducing fine process equipment such as a photo process etching device. The other is the process established to provide a constant conversion efficiency despite changes in illuminance. The process permits the obtaining of almost equal conversion efficiency in the interior of a room (200 luxes) and right under the solar light and to provide a constant charge of electricity to secondary cells and condensers regardless of whether indoors or outdoors.

The a-Si solar cells in new shapes are used in the (Alba) brand Hattori Seiko, analog quartz wristwatches. Fuji Electric expects other applications beyond wristwatches, to cameras, radio receivers, and memory backups for audio and home appliances.

Sharp Corporation
Technical Tie-up With ECD of the United States

Sharp enjoys a 97-percent domestic market share of single crystal solar cells as far as the a-Si solar cell is concerned. Since the development of the ground solar cell (single crystal) in 1963, Sharp has led the industry in the development of space, ground, and civilian single crystal solar cells. It is now delivering single crystal solar cells as a power source for artificial satellites, lighthouses, observatories, road information guideposts, and defense-related equipment.

The company set up a collaborative relationship with ECD of the United States on a-Si solar cells in June 1982, and established Sharp ECD Solar, a joint venture company, to enter the a-Si solar cell market. The action was designed to build up development and production systems of a-Si solar cells having outstanding features through the combination of the a-Si solar cell technology of ECD with the Sharp's solar cell technology which had been built up over a long period of time.

The special feature of the a-Si solar cell production by the joint venture company is that the plasma CVD method using fluoric gas is adopted in the process. Since the F-Si bond energy is larger than that of the existing H-Si bond, its heat-resistance and weatherproof quality are high so their a-Si solar cells will show less deterioration due to irradiation. Its two-layer tandem structure allows an increase in the sensitivity to the wideband wavelength of light and the absorption of solar energy efficiently.

Sharp predicts that while the a-Si solar cells will be leading the solar cells for civilian indoor use, the single and polycrystal silicon solar cells will be central in the applications for outdoor use. Based on the perception that the a-Si solar cells and single crystal solar cells will be growing in their respective application in accordance with their special features, the company "will aim to become a general solar cell manufacture" (Sharp executive).

Taiyo Yuden Co., Ltd.
Transparent Tin Oxide Electrodes Adopted

Taiyo Yuden has independently developed a-Si solar cells incorporating transparent tin oxide electrodes and presently ranks fourth in manufacturing. In a joint development with the Electrotechnical Laboratory the company succeeded in producing a solar cell with a 10.26 percent conversion efficiency (2-mm^2) in 1983.

This cell is structured as follows: A rough-surfaced tin oxide film with a large diameter is formed on an a-Si layer under which an indium oxide film containing tin is attached to the portion of electrode. With this structure the light entering from outside is dispersed first by the tin oxide film and then enters the a-Si layer. Since the refractive index of the electrode portion made of indium oxide is smaller than that of the a-Si layer, the light approaching the electrode from the a-Si layer is totally reflected at the

boundary layer, being unable to make its way out, thus light containment is achieved.

Nonelectric Manufacturers Making Entry

Komatsu Electronic Metals (head office: Hiratsuka City, Kanagawa Prefecture), a member of the Komatsu Ltd. group, a construction manufacturer, is the world's sole manufacturer producing silicon semiconductors from monosilane gas and engaged in making single crystal solar cells. It is participating in studies under the NEDO project on the technology to produce monosilane at low cost, which is required in making a-Si solar cells.

The special features of the a-Si solar cells made by Komatsu are: 1) using disilane gas as a raw material, high-speed amorphous lamination, 2) improving the quality of the a-Si film through the fine control of the condition of plasma, and 3) reducing the reflection losses of incident light by improving transparent electrodes. Presently, a 10.7 percent conversion efficiency per 2-mm^2 is achieved.

The participants in the NEDO project from the material makers' side are Kyocera Corp. and Teijin Ltd.

Kyocera, which is a leader in the production of polycrystals, aims to develop a high efficiency a-Si solar cell with a ceramic wafer. In order to increase a-Si solar cell production its Yokaichi Plant (Yokaichi City, Shiga Prefecture) has changed its production system to raise the output to 1 million units monthly.

Teijin is stepping up the development of an a-Si solar cell using a flexible high molecular film (polyamide film) as a wafer.

The companies which have not participated in the NEDO project, but made successful entry into the field of a-Si solar cells are Showa Shell Sekiyu K.K., Kanegafuchi Chemical Industry Co., Ltd., and TDK Corporation.

Showa Shell Sekiyu, putting its energies into the nonoil sector, last year sold solar cells (single crystal) in the amount of Y600 million and aims this year at Y1 billion. With respect to the a-Si solar cell, it intends to construct a pilot plant in 2 years at its central laboratory in Kanagawa Prefecture through the introduction of technology from Arco Solar, a subsidiary company of Atlantic Richfield, a leading oil company.

Kanegafuchi Chemical has developed an amorphous silicon carbide amorphous silicon (SiC: H/a-Si:H) heterobond solar cell by using technology available only to chemical companies [sentence as published]. While the basic research on the solar cells is conducted at its central laboratory in Kobe City, the research on mass production technology is carried out at its electronic material development laboratory in Ohotsu City.

Semiconductor Energy Laboratory (head office in Setagaya-Ku, Tokyo), an affiliate of TDK, achieved in November 1984 a 9.41 percent conversion

efficiency per 10 cm^2 by adopting the separate formation method and laser-scribing method. These two new methods have obtained patents in the United States.

Theoretically Highest Conversion Efficiency Is 24.5 Percent

Although every maker is making desperate efforts in developing a-Si solar cells, there are some experts who predict a bright future for the a-Si solar cells.

The first reason is the difficulty in raising conversion efficiency. The present conversion efficiency of the a-Si cells is a little over 8 percent for a large area of 10 cm^2 and a little over 11 percent for a small area of some 2 mm^2. They find it difficult to catch up with single crystal and polycrystal cells showing 13-16 percent.

The second reason is that exposure to a strong light such as direct rays of the sun leads to the destruction of the system in part and subsequently to the further reduction of conversion efficiency. There is the view that unless these two points are rectified, the a-Si solar cells can hardly expand power source applications beyond the current applications for civilian use such as electronic calculators and watches.

However, the theoretically predictable highest conversion efficiency of the a-Si solar cell is 24.5 percent. Yukinori Kuwano of the central laboratory of Sanyo Electric has declared that even a large area a-Si solar cell would be able to hit the 20-percent mark by the beginning of the 1990's.

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NUCLEAR DEVELOPMENT

TEST FACILITY FOR FUSION REACTOR MATERIALS DEVELOPMENT COMPLETED

Tokyo GENSHIRYOKU SANGYO SHIMBUN in Japanese 6 Feb 86 p 7

[Text] On 30 January the National Research Institute for Metals announced completion, at its Tsukuba branch in Ibaraki, of a light ion irradiation creep test facility which will use a small cyclotron for research and development of new materials for fusion reactors. The facility will be the world's first devoted solely to materials radiation using an accelerator. It will be possible to conduct pulling and twisting tests on materials while they receive continuous irradiation with stable ions for a period of 100 hours. It is hoped it will produce important results for the development of internal structural materials for working fusion reactors.

The internal structural materials in fusion reactors are subjected to extremely harsh conditions of use in that they are bombarded and weakened by high-energy neutrons generated by the fusion reaction. In fission reactors and even in fast breeder reactors, the energy of neutrons is 8 MeV at the highest, and averages 1 MeV. But in a fusion reactor it reaches 14 MeV.

Consequently, while working-level neutron irradiation tests for a fast breeder reactor can be done in a materials irradiation reactor, if one attempts the same tests for a nuclear fusion reactor, even the RTNS-II device at Livermore National Laboratory in the United States, the world's highest energy device, can only provide irradiation tests at one-thousandth or one ten-thousandth the radiation density of a working-level fusion reactor.

"Creep" refers to the progress of deformation when stress is applied to materials in a high temperature state. Under neutron irradiation, not only does the speed of deformation increase, but the temperature range at which creep occurs tends to extend to lower temperatures.

The completed device, which will irradiate with protons at 17 MeV to give the same irradiation effect as neutrons at 14 MeV, will be used in the development of materials with superior creep characteristics.

The small cyclotron is based on the Japan Steel Works product which is widely used in medicine, but has such improvements as (1) a long-lasting ion source, (2) materials with low propensity for becoming radioactive, and (3) computer control.

With regard to the development of nuclear fusion in Japan, the JT-60 critical plasma test device of the Japan Atomic Energy Research Institute is intended to achieve critical plasma conditions during 1987; the subsequent development of such things as a nuclear fusion test reactor will face the major problem of how to set neutron irradiation conditions. The development of new materials which can withstand a hostile neutron environment will become more and more necessary.

It is therefore the policy of the National Research Institute for Metals to conduct research including (1) improvement of radiation brittleness of high-nickel, eduction-strengthened alloys, (2) development of revolutionary materials such as single-crystal alloys and noncrystalline alloys, (3) comparison of advantages and disadvantages of ferrite-martensite heat-resistant steels and austenite stainless steels, (4) evaluation of helium brittleness behavior and (5) mutual irradiation of the cyclotron and the "Joyo" working fast breeder reactor.

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TELECOMMUNICATIONS

FAIR TRADE COMMISSION REPORTS ON VALUE ADDED NETWORK

Tokyo JECC NEWS in Japanese 1 Jun 85 p 2

[Text] The Fair Trade Commission recently conducted a survey on the effect of the Antimonopoly Act on the information-communication industry field attendant to the privatization of the Nippon Telegraph and Telephone Public Corp., and has prepared a report entitled "The Ideal Way and Theme of Competition Policy in the Telecommunications Industry Field." The report includes a section giving the results of a questionnaire on how the principal firms will move against telecommunications conducted among private firms.

The survey was conducted from the end of January to February this year on 450 general firms (listed firms) and 100 information industry firms (firms ranking high in sales), centering particularly on how they intend to move against the VAN business. Response to this came from 162 general firms (broken down into 70 manufacturing firms, 64 financial and distribution firms, and 28 service industry firms) and 33 information industry firms (of which 9 have already reported to the competent office on their entry into small-scale VAN business).

This paper excerpts a part of the results of the questionnaire and introduces the present situation of the principal firms' moves against the VAN business.

Participation in VAN Business

1. Intention of participation in VAN business

Tables 1 and 2 show the results of the questionnaire on the intention of participation in the VAN business.

Of the 33 information industry firms, 9 (27.3 percent) are already operating small-scale VAN. Further, four firms (12.1 percent) plan to start the VAN business within 3 years.

In the general firms, 47 (29 percent) of the 162 answered that they have a plan to conduct the VAN business, expressing their intention to participate in the VAN business. However, many are still in the planning stage and only 14 firms (8.6 percent) plan to conduct this business within 3 years.

Table 1. Intention for VAN (Information Industry)

Now operating small-scale VAN	9 firms (27.3 percent)
Planning to conduct VAN within 1 year	3 firms (9.1 percent)
Planning to conduct VAN within 2 to 3 years	1 firm (3.0 percent)
In planning stage	13 firms (39.4 percent)
Having no plan	7 firms (21.2 percent)
Total	33 firms (100 percent)

Table 2. Intention of Participation in VAN Business (General Firms)

Having a plan to conduct VAN business	47 firms (29 percent)	Within 1-3 years	14 firms (8.6 percent)
		Still in planning stage	33 firms (20.4 percent)
Having no plan	115 firms (71 percent)		
Total	162 firms (100 percent)		

2. Motive for participation in VAN business and prospect for profit

To examine the motive for participation in the VAN business with regard to the general firms, many firms say that this business will serve to activate and expand their own existing business and will lead to effective utilization of their own data communications network, and few think of it as a profit-making business.

The general firms think of the VAN business not as a profit-making business field, but rather as a means of developing their existing business or as a measure to promote the efficiency improvement of their own data communications network. This consciousness appears also in their prospect for profit, and it seems that many of the general firms that are to participate in the VAN business do not expect very much of it in terms of profit-making.

On the other hand, there are also many firms in the information industry that say they cannot expect a profit for the time being, but the greater part of the firms expect that they can make a profit by VAN.

Table 3. Motive for Participation in VAN (General Firms) N = 14

(Firms)

	1. Quite strongly motivated	2. Somewhat strongly motivated	3. Not motivated	No reply
(1) VAN business serves for activation and expansion of existing business	10	1	0	3
(2) VAN business leads to effective utilization of own data communications network	7	4	2	1
(3) Because of request from related firms	3	6	2	3
(4) Because possibility as profit-making business is strong	3	2	5	4

Table 4. Prospect for Profit From VAN

(Firms)

	General firms N = 14	Information industry N = 13	Total
(1) Expect considerable profit as a new business	1	0	1
(2) Expect it to fully pay as a business, although profit not likely to be very large	3	6	9
(3) Consider that profit cannot be expected for the time being	6	5	11
(4) Do not think of this business as a profit-making business from the beginning	0	2	2
No reply	0	2	2

Table 5. Operating Form of VAN

(Firms)

	General firms N = 14	Information industry N = 13	Total
1. To operate by establishing a subsidiary of their own firms	3	0	3
2. To operate by establishing a firm by joint investment with other firms	5	1	6
3. To industrialize and operate in their own firms	6	11	17
No reply	0	1	1

Plan for VAN Business

1. Operating form of business

To examine the operating form of business, almost all firms of the information industry answered that they will industrialize and operate it in their own firms but many of the general firms say they will operate it by establishing a firm through joint investment with other firms.

2. Service area of VAN

As for the service area of VAN, the majority of general firms say they aim at an unlimited nationwide area. Against this, the information industry cited a variety of VAN service areas showing quite a positive attitude toward the expansion of the network abroad in comparison with the general firms.

3. Operating affairs of VAN

In the general firms, many cited processing of business information, mailbox, and provision of data base. In the information industry, on the other hand, many firms cited format conversion, speed conversion, protocol conversion, etc., proving that they are planning to provide wide-ranging services centering on technical service with a general-purpose nature.

4. VAN business plan

To examine the users at whom VAN business aims, the number of firms saying they aim at unspecified firms is largest. In the general firms, however, many say they aim at mainly firms in capital affiliation with their own firms, indicating their lateral view that they are considering VAN in terms of strengthening relations among their affiliated firms. In the information industry, on the other hand, there are also many firms which say they are conducting limited VAN centering on specific industries.

Table 6. Characteristics of Users

(Firms)

	General firms N = 14	Information industry N = 13	Total
1. Mainly firms in capital affiliation with their own firms	6	2	8
2. Mainly specific industries	1	5	6
3. Aiming at unspecified firms	6	5	11
4. Others (concretely)	1	0	1
No reply	0	1	1

The number of firms expected to participate in VAN of their own firms varies considerably with the firm, being about 10 to 3,000 in the general firms and about 5 to 500 even in the information industry. This depends on whether they consider unspecified firms or their affiliated firms and specific industries as to the aims of their VAN.

Next, as to the types of businesses to participate in VAN, many firms cite transportation and wholesale in the general firms and financial interests and distribution in the information industry.

The investment in VAN development cited is about Y300 million to Y20 billion in the general firms and about Y600 million to Y60 billion in the information industry. The sales goal cited is Y5 million to Y3 billion and Y5 million to Y100 billion, respectively. However, the sales goal is not necessarily large in proportion to the amount of investment, and their views on demand for the VAN business appear to differ considerably.

Next, with regard to the technical development necessary for the VAN business, many firms intend to conduct domestic development, including development by their own firms, both in the hardware and software fields. This reflects the fact that the standard of Japan's technology is already at a high level, but particularly with regard to application software it is also felt that the results of the survey indicate that it is impossible to cope with the needs of users by a mere introduction of technology from abroad.

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